

Genome sequence of *Streptomyces antioxidans* MUSC 164^T isolated from mangrove forest

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Abstract: Members of *Streptomyces* genus are well known to produce bioactive compounds of various structures^[1-3]. Having a complicated development life cycle with the ability to form spores, *Streptomyces* species are ubiquitous in nature and can be found in interesting places like deep sea, hot springs and also mangrove forest^[3-11]. *Streptomyces antioxidans* MUSC 164^T was originally isolated from mangrove forest in the east coast of Peninsular Malaysia and has been deposited at two culture collection centres (=DSM 101523^T = MCCC 1K01590^T)^[12,13]. After rounds of *in vitro* screening using human neuronal cell line (i.e. SH-SY5Y), the extract of MUSC 164^T was found to possess significant neuroprotective effect against hydrogen peroxide^[13]. Here, a high quality genome sequence of MUSC 164^T is reported, while its genome potential to produce pharmaceutically important compounds is also discussed.

Keywords: *Streptomyces*; antioxidant; genome; mangrove; actinobacteria; next generation sequencing (NGS)

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Short Introduction

As a Gram-positive bacterium, *Streptomyces antioxidans* MUSC 164^T forms yellowish white aerial mycelium and brilliant greenish yellow substrate mycelium on yeast-malt (ISP 2) agar^[13]. Based on polyphasic study, this strain was concluded as a novel species belonging to

the genus *Streptomyces*, displaying with highest partial 16S rRNA gene sequence similarity with type strain *Streptomyces javensis* NBRC 100777^T (99.6 % sequence similarity), *Streptomyces yogakartensis* NBRC 100779^T (99.6 %) and *Streptomyces violaceusniger* NBRC 13459^T (99.6 %). The methanolic extract of MUSC 164^T showed significant antioxidative and neuroprotective activities

against hydrogen peroxide. As an attempt to further explore its bioactive potential, the strain was selected for genome sequencing.

Data description

Genomic DNA of MUSC 164^T was extracted using Masterpure TM DNA purification kit (Epicentre, Illumina Inc., Madison, WI, USA) before performing RNase (Qiagen, USA) treatment^[14,15]. DNA Quality was evaluated using NanoDrop spectrophotometer (Thermo Scientific, Waltham, MA, USA) and a Qubit version 2.0 fluorometer (Life Technologies, Carlsbad, CA, USA). Whole-genome shotgun sequencing of strain MUSC 164^T was carried out on Illumina MiSeq platform with MiSeq Reagent Kit 2 (2 × 250 bp; Illumina Inc., Madison, WI, USA), and it generated 8,806,977 raw reads. Subsequently, ambiguous nucleotides, reads that are shorter than 50 bps and low quality reads were removed from the data. The draft genome was assembled with CLC Genomics Workbench version 5.1 (CLC bio, Denmark). Contigs with at least 200 bp and 30-fold coverage were selected for gene prediction and annotation. The bacteria identity was also checked by local BLAST against NCBI prokaryotic 16S rRNA database. Bacteria gene coding sequence (CDS) was predicted from the draft genome using Prodigal (version 2.6.1)^[16]. Gene annotation was performed by local BLAST of translated predicted CDS against NCBI-nr database and also on Rapid Annotation using Subsystem Technology (RAST) server^[17]. Presence of rRNA and tRNA genes were detected using RNAmmer and tRNAscan SE version 1.21^[18,19].

A total of 282 contigs were generated with N50 size of 111,730 bp. The assembled genome size of MUSC 164^T contains 9,118,065 bp, with an average genome coverage of 141-fold with a G + C content of 71.5 % (Table 1). The whole genome project was deposited at DDBJ/EMBL/GenBank under accession LAKD00000000. The version described in this paper is the second version,

LAKD02000000. It is composed of 282 contigs and there were 7,214 protein coding genes (out of a total of 7,620 predicted genes) (Figure 1).

Table 1. General features of *Streptomyces antioxidans* MUSC 164^T draft genome.

Attribute	Value
Genome size (bp)	9,118,065
DNA G+C (bp)	6,516,852
DNA scaffold	282
Total genes	7,620
Protein coding genes	7,214
RNA genes (5S, 16S, 24S)	4, 4, 2
Pseudo genes	331
CRISPR repeats	7

Using antiSMASH to detect biosynthetic gene clusters, the analysis revealed presence of 11 biosynthetic gene clusters which exhibited more than 70 % similarities with known gene clusters^[20]. Out of which, five clusters were associated with production of non-ribosomal peptides, including coelichelin, roseoflavin and paenibactin. Interestingly, the genome of MUSC 164^T revealed potential production of valuable siderophores like desferrioxamine B. In short, the availability of genome sequence for *Streptomyces antioxidans* MUSC 164^T has suggested its genome potential and prompted further improvement work (e.g. media optimization, strain improvement) to fully invoke the bioactive potential of the strain, particularly for production of valuable pharmaceutical compounds like desferrioxamine B.

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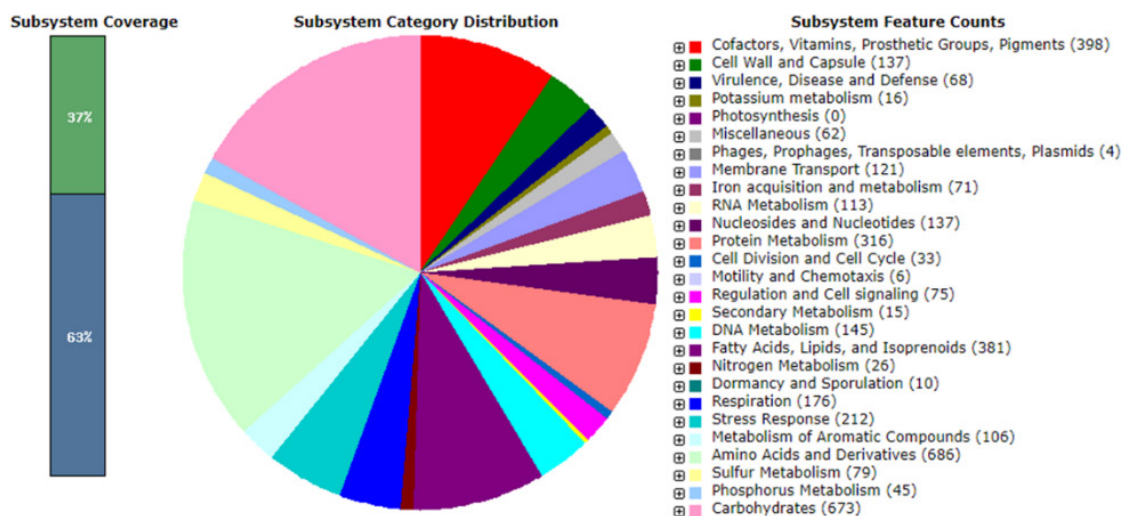


Figure 1. Subsystem category distribution of *Streptomyces antioxidans* MUSC 164^T (based on RAST annotation server).

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Conflict of Interest

The authors declared that there is no conflict of interest.

References

- Berdy J. Bioactive microbial metabolites. *J Antibiotics* 2005; 58: 1–26.
- Ser HL, Tan LTH, Law JWF, *et al.* Focused review: Cytotoxic and antioxidant potentials of mangrove-derived *Streptomyces*. *Front Microbiol* 2017; 8: 2065.
- Subramani R, Aalbersberg W. Marine actinomycetes: An ongoing source of novel bioactive metabolites. *Microbiol Res* 2012; 167(10): 571–580.
- Kamjam M, Sivalingam P, Deng, Z, *et al.* Deep sea actinomycetes and their secondary metabolites. *Front Microbiol* 2017; 8: 760.
- Yang N, Song F. Bioprospecting of novel and bioactive compounds from marine actinomycetes isolated from South China Sea sediments. *Curr Microbiol* 2017: 1–8.
- Al-Dhabi NA, Esmail GA, Duraipandiyar V, *et al.* Isolation, identification and screening of antimicrobial thermophilic *Streptomyces sp.* Al-Dhabi-1 isolated from Tharban hot spring, Saudi Arabia. *Extremophiles* 2016; 20(1):79–90.
- Duan YY, Ming H, Dong L, *et al.* *Streptomyces calidiresistens sp. nov.*, isolated from a hot spring sediment. *Antonie van Leeuwenhoek* 2014; 106(2): 189–196.
- Tan LTH, Ser HL, Yin WF, *et al.* Investigation of antioxidative and anticancer potentials of *Streptomyces sp. MUM256* isolated from Malaysia mangrove soil. *Front Microbiol* 2015; 6: 1316.
- Zainal N, Ser HL, Yin WF, *et al.* *Streptomyces humi sp. nov.*, an actinobacterium isolated from soil of a mangrove forest. *Antonie van Leeuwenhoek* 2016; 109(3): 467–474.
- Ser HL, Palanisamy UD, Yin WF, *et al.* *Streptomyces malaysiense sp. nov.*: A novel Malaysian mangrove soil actinobacterium with antioxidative activity and cytotoxic potential against human cancer cell lines. *Sci Rep* 2016; 6: 24247.
- Ser HL, Zainal N, Palanisamy UD, *et al.* *Streptomyces gilvigriseus sp. nov.*, a novel actinobacterium isolated from mangrove forest soil. *Antonie van Leeuwenhoek* 2015; 107(6): 1369–1378.
- Lee LH, Zainal N, Azman AS, *et al.* Diversity and antimicrobial activities of actinobacteria isolated from tropical mangrove sediments in Malaysia. *Scientific World J* 2014; 2014: 1–14.
- Ser HL, Tan LTH, Palanisamy UD, *et al.* *Streptomyces antioxidans sp. nov.*, a novel mangrove soil actinobacterium with antioxidative and neuroprotective potentials. *Front Microbiol* 2016; 7: 899.
- Ser HL, Tan WS, Ab Mutalib NS, *et al.* Draft genome sequence of mangrove-derived *Streptomyces sp. MUSC 125* with antioxidant potential. *Front Microbiol* 2016; 7: 1470.
- Ser HL, Tan WS, Ab Mutalib NS, *et al.* Genome sequence of *Streptomyces mangrovisoli MUSC 149^T* isolated from intertidal sediments. *Braz J Microbiol* 2018; 49(1): 13–15.
- Hyatt D, Chen GL, Locascio PF, *et al.* Prodigal: Prokaryotic gene recognition and translation initiation site identification. *BMC Bioinform* 2010; 11: 119.
- Aziz RK, Bartels D, Best AA, *et al.* The RAST Server: Rapid annotations using subsystems technology. *BMC Genomics* 2008; 9: 75.
- Lowe TM, Eddy SR. tRNAscan-SE: A program for improved detection of transfer RNA genes in genomic sequence. *Nuc Acids Res* 1997; 25: 955–964.
- Lagesen K, Hallin P, Rodland EA, *et al.* RNAmmer: Consistent and rapid annotation of ribosomal RNA genes. *Nuc Acids Res* 2007; 35: 3100–3108.
- Blin K, Medema MH, Kottmann R, *et al.* The antiSMASH database, a comprehensive database of microbial secondary metabolite biosynthetic gene clusters. *Nuc Acids Res* 2016; 45: D555–D559.